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Expression of Beta-Adrenergic Receptor in Glioma LN229 Cells and Its Effect on Cell Proliferation

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Abstract. To investigate the expression of beta-adrenergic receptor(β-AR) in glioma cells and the effect of blocking β-adrenergic receptors on glioma cell proliferation. Methods: The expression of beta-adrenergic receptor in glioma LN229 and U251 cells were detected by real time PCR and Western Blotting. The proliferation of glioma cells was analyzed by MTT assay after they were exposed to norepinephrine and propranolol(non-selective blocker of beta adrenergic receptors) alone, or in combination, respectively. The LN229 and U251 cells both expressed beta 1 and beta 2 adrenergic receptors, but beta2-receptor showed a relatively strong expression while beta1-receptor showed a relatively weak expression in LN229cells. The proliferation of glioma cells was significantly inhibited by propranolol which acts as a nonspecific blocker of beta-adrenergic receptors, with a certain time and concentration-dependent matter toward higher inhibition, and the differences had statistically significance (all P <0.05). The proliferation LN299 cells were significantly increased after norepinephrine, which was in a concentration-dependent manner antagonized by propranolol co treatment (P <0.05). Both adrenergic beta1 and beta2 are expression of glioma cells, and non-selective blockage of beta adrenergic receptor can effectively inhibit the proliferation of glioma cells.

Introduction

Brain glioma is the most common primary central nervous system tumors. As a result of psychological fear, tumor patients with long-term psychological stress, so that the body's neuroendocrine system continues to release stress hormones, such as epinephrine (EPI), norepinephrine (NE) and so on[1].It is found that norepinephrine is closely related to the occurrence, development and metastasis of various tumors [2-4]. The purpose of this study was to investigate the expression of β1 and β2 adrenergic receptors in glioma cells, to investigate the effect of non-selective beta adrenergic receptor blocker propranolol on its proliferation of glioma cells.

Materials and Methods

Norepinephrine, Propranolol, and MTT (Sigma); Fetal bovine serum FBS, DMEM medium(Gibco); The glioma cell line LN229 and U251 were came from the American Type Culture Collection (ATCC);β1, β2 adrenoceptor rabbit monoclonal antibody, and anti-β-actin were came from Santa Cruz; TRIZOL(Invitrogen); Fast Quant RT kit and Real-time quantitative PCR kit (TOYOBO FEQ).

Cell Culture. LN229 and U251 cells were cultured in DMEM medium containing 10% fetal bovine serum at 37 ℃, 5% CO_2 incubator. The cell density was 8×10^5 after digested by trypsin in logarithmic growth phase, the cells were inoculated into a cell culture plate, when the cells were adhered and the medium was discarded and high glucose DMEM medium was supplemented with different concentrations of norepinephrine and/or different concentrations of propranolol. After the intervention of 24、48 and 72 h, respectively. The cells were used in the following experiments. Each well was repeated three times, and the experiment was repeated three times.

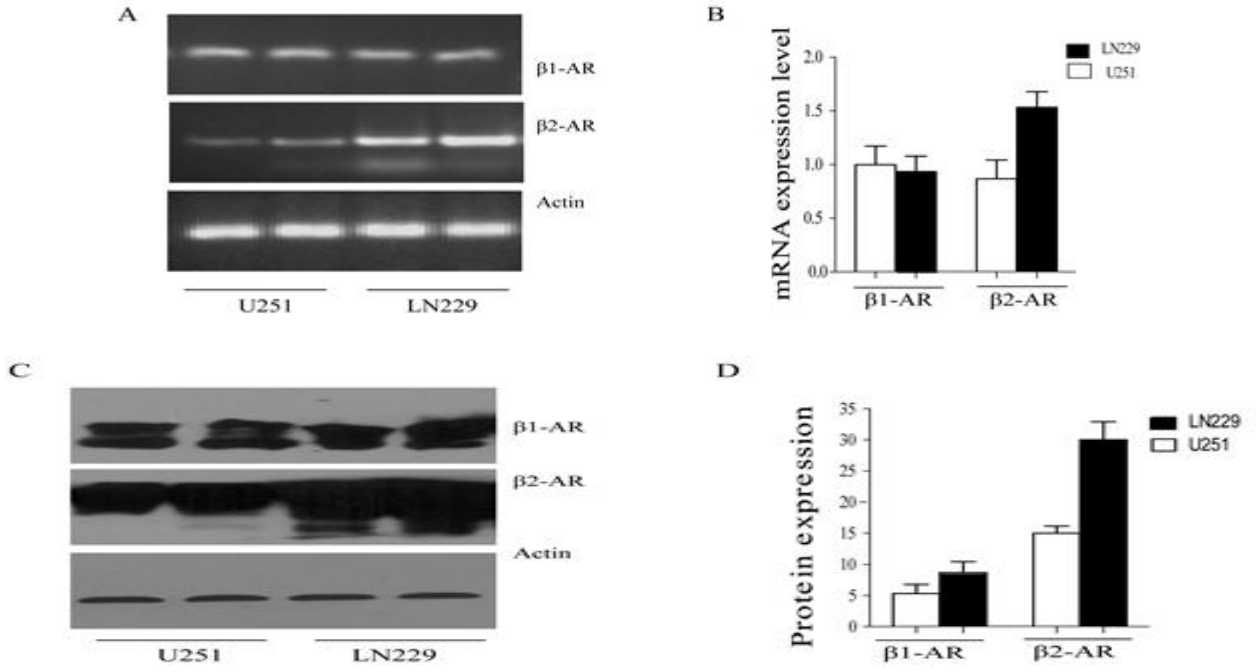
The Expression of β1 and β2 Adrenergic Receptor mRNA in Glioma Cells. The total RNA was extracted from glioma cells for reverse transcription, followed with the reverse transcription kit instructions, and then amplified by PCR. The primer were β1(395bp)Forward:5′TTGGGACGTTGG, ACAGCAGG3′Reverse: 5′GGCAGTGTGTGTGTCGTGTGAGA3′, β2 (563bp)Forward: 5′GTGAG TCCTTGCCCTGCTTC3′Reverse: 5’ TCCCCGTGCATCTTTCTGT3′, β-Actin(187bp) Forward: 5′AGGGGCCGGACTCATCGTA 3′, Reverse: 5′GAGCTCACCATTCACCATCTTGTC 3′. Amplification conditions: 95 ℃ 30s, denaturation of 95 ℃ 5s, annealing of 60 ℃ 30s, extension of 72 ℃ 50s, 35 cycles and then extended 72 ℃ 2min, cooled to 4 ℃. Each design 3 replicates hole, Then the relative expression of mRNA was calculated with 2-Δ Δ Ct.

The Expression of β1, β2 Adrenergic Receptor Were Detected by Western Blotting. The total protein was extracted and subjected to polyacrylamide gel electrophoresis , then transferred to NC membrane, Closed with 5% nonfat dry milk for 1 hour, Add an anti beta 1 adrenergic receptor (1: 2000), beta 2 adrenergic receptor (1: 5000) and Actin (1: 5000), respectively, and then overnight at 4℃, wash with TBST 3 times, each time for 10 minutes, Goat anti-rabbit secondary antibody (1: 5000) at room temperature for 1.5 hours, wash with TBST 3 times, each time for 10 minutes, ECL substrate color, and the gray value was analyzed.

The Cell Proliferation Was Detected by MTT Colorimetric Assay. Glioma LN229 cells were treated with 0、0.1、1、10 μmol/L norepinephrine and/or propranolol (0、0.1、1、10μmol/L),Cells without drug as control group, The average absorbance A value of each group was detected after 24、48 and 72h, respectively. The cell proliferation inhibition rate (%)= (1 - the average absorbance of drug group A value / average absorbance of the control group A value) × 100%.

Statistics Analysis. SPSS 21.0 statistical software was used to analyze the data as mean ± standard deviation ($\bar{x} \pm s$), and the mean values were compared by t test, P <0.05 was statistically significant.

Results

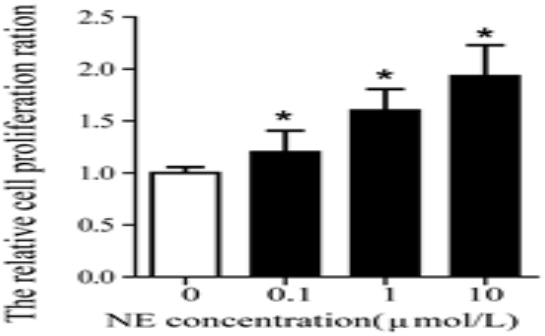


A. RT-PCR result; B. qPCR result; C. Adrenergic receptors expression; D. Protein analysis

Figure 1. Expression of β receptor in glioma cells

The Expression of Adrenergic Receptors in Glioma Cells. The expression of β1 receptor and β2 receptor in U251 and LN229 cell lines were detected by PCR and western blot. The results showed that both glioma cells expressed β1 and β2 receptors . The beta 2 receptor of LN229 cells was slightly higher than that of beta 1 receptor, see Fig. 1.

Effect of NE on Proliferation of Glioma LN229 Cells. With the increase of NE concentration, the proliferation of LN229 cells was dose dependent. Compared with the blank control group (NE = 0μmol / L), Compared with the blank control group (NE=0 mol/L), 0.1 mol/L, 1 mol/L and 10 mol/L NE group had significant statistical significance (P <0.05). The results showed that NE could promote the proliferation of LN229 cells, and the dose-effect relationship is good, as shown in Figure 2. Because the experimental window of 10μmol / L NE group is larger, suitable for further



experiments, so the experiment selected 10μmol / L NE, see Fig. 2.

Figure 2. Effect of different concentrations of NE on the proliferation of LN229 cells

Inhibitory Effect of Propranolol on the Proliferation of LN229 Glioma Cells. There was no significant difference in cell growth inhibition rate between LN229 cells treated with different concentrations of propranolol at 24 h（P>0.05).But there was a significant inhibitory effect on cell proliferation at 48 h, and there was a concentration dependent relationship (P<0.05). The inhibitory rate of LN229 cells was 18.54%, at the concentration of 0.1 μmol / L; 36.41% at 1 μmol / L; and 58.23% at 10 μmol / L. The inhibitory effect of propranolol on the growth of LN229 cells was concentration and time-dependent. Inhibition effect of LN229 cells by different concentrations of propranolol was the strongest at 72 h (Table 1).

Tablel 1 Rates of growth inhibition of LN229 cells after exposure to different concentration propranolol for different times [%]

Propranolol [μ mol/L]	Time (h)		
	24	48	72
0.1	12.70 ± 3.46	18.54 ± 3.14*	31.39 ± 6.67*
1.0	22.81 ± 5.18#	36.41 ± 2.45*	53.85 ± 5.09*
10	13.37 ± 3.37##	58.23 ± 6.53*##	80.16 ± 2.84*#

Note: *P <0.05 vs. the same concentration group at 24h；#P <0.05， ##P <0.01.vs. 0.1μmol/L group at the same time point.

The Effect of Combined Application of Propranolol and Norepinephrine on Proliferation of Glioma LN229 Cells. When combined with propranolol and norepinephrine, the proliferative activity of cells was lower than that of norepinephrine alone, but the proliferative activity was higher than that of propranolol alone (all P <0.05), see Fig. 3.

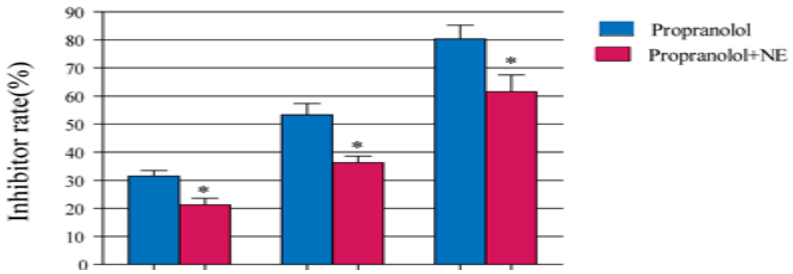


Figure 3. Influence of norepinephrine and propranolol alone or combination on proliferation of glioma LN229 cell

Discussion

Stress is the systemic non-specific adaptation reaction which occurs when the body is stimulated by various internal and external environmental factors and social and psychological factors. After the stress, sympathetic-adrenal medulla axis excites, plasma epinephrine and NE concentration increased rapidly, cause the physiological reaction [5,6]. Norepinephrine is closely related to the development of breast cancer, pancreatic cancer, nasopharyngeal carcinoma and other malignant tumors. Adrenergic receptors are divided into alpha receptors, beta receptors, which are divided into beta 1, beta 2 adrenergic receptor. Recent studies have shown that β-adrenergic receptors were involved in tumor proliferation, invasion, migration and other biological behavioral regulation [7-9], and play an important role in tumor progression. The experimental results show that two kinds of beta adrenergic receptors were expressed in glioma cell line LN229 and U251.

In recent years, it has been found that the stress response is closely related to the prognosis of cancer patients. Norepinephrine plays a significant role, NE mainly affects the invasion and migration of a variety of tumor cells, such as ovarian cancer, pancreatic cancer, breast cancer, nasopharyngeal carcinoma, colon cancer and so on through the β receptor, alpha receptor in which does not play a role [10,11]. Propranolol is a clinically used β-blockers that have been used for decades. It can widely block beta 1, beta 2 receptor, and there is no intrinsic sympathomimetic activity. At present, studies have pointed out that the stress hormone may be through beta adrenergic receptor to promote the invasion of a variety of tumor cells, the application of beta blocker propranolol can be a good reversal of stress hormone-induced tumor invasion, but the effects of stress hormones on the proliferation of human glioblastoma cells is unclear. In this study, we tested the inhibitory effect of propranolol on proliferation of LN229 glioma cells, which blocks both β1 and β2 receptors. MTT assay showed that propranolol inhibited the proliferation of glioma cells. The inhibitory effect of protonol on glioma cells was time and concentration-dependent trend. Norepinephrine promoted the growth of LN229 cells, while propranolol antagonized the proliferation of glioma cells induced by norepinephrine.

In summary, the results of this study show that the β-adrenergic receptor blocker propranolol can inhibit NE-induced proliferation of human glioblastoma. This study provides a theoretical basis for the treatment of glioma propranolol as adjuvant, especially propranolol can be used as an aid in glioma therapy in the acute stress state during perioperative period.

Acknowledgements

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